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Single- and multi-species groups: A descriptive study of cattle and broiler behaviour on pasture

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ABSTRACT

Farming with more than one livestock species has been discussed as being more sustainable compared to specialised production systems. Some potentially positive effects of multi-species livestock farming can only occur when different species share space, e.g., by co-grazing. To date there is limited research on the behaviour of animals in multi-species livestock groups. If co-grazing is adopted more widely, it is important to investigate its effect on the animals' behaviour and subsequently their welfare. One of the most prevalent species combinations in a survey of 126 multi-species livestock farms was cattle and poultry. Therefore, we aimed to describe and quantify inter-species interactions between young cattle and broiler chickens when co-grazing and descriptively compare intra-species interactions as well as maintenance, comfort and social behaviours between single- and multi-species groups of cattle and broilers on pasture. Additionally, we assessed fearfulness in broilers grazing with or without cattle as measured by Tonic Immobility (TI), an Inversion test and a Novel Object test. Across five replicates we observed three groups in each six-week cycle: One single-species group of ten cattle, one single-species group of 54–61 broilers, and one multi-species group of ten cattle and 54–61 broilers. Once per week the multi-species group was observed for 120 min using behaviour sampling to quantify the occurrence of inter- and intra-species interactions. On two other days per week, single- and multi-species groups were observed in a balanced order using continuous focal animal sampling to assess frequency and duration of maintenance, comfort and social behaviours for ten animals per species for 6 min each. During all observations scan sampling was performed every 6 min to count the number of visible animals per pasture sector. Across observation methods, two independent observers achieved high agreement for most behaviours. Inter-species interactions occurred two to three times per hour and ten animals per species, whereas intra-species interactions occurred between 18 and 28 times per hour and ten cattle or broilers. The most frequently observed inter-species interactions were cattle displacing broiler and broiler approaching cattle. More generally, maintenance, comfort and social behaviours as well as measures of fearfulness did not differ between animals in single- and multi-species groups, but the variation was high across observations. These findings should be verified with a greater number of animals and groups to be more applicable to commercial settings, but we conclude tentatively that co-grazing did not affect cattle or broilers negatively.

1. Introduction

Multi-species livestock farming, after a decline due to specialisation on a single animal species or production type, experiences renewed interest as it may contribute to a more sustainable agricultural production (Martin et al., 2020). Several benefits have been proposed: better resource use efficiency due to, for example, the possibility of using pasture with two species with different feed preferences; health and performance benefits for the animals in rotating or simultaneous grazing systems, for example due to a decreased parasite load; and increased economic stability (for review on multi-species livestock farming see Martin et al., 2020).

Some benefits may only occur when two livestock species share one pasture at the same time, which may influence the animals' behaviour

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and subsequently their welfare. In extensive or remote agricultural systems, simultaneous grazing of a small number of animals of one species is often used to protect a larger number of animals of another animal species from predation, e.g., one llama with a flock of sheep (Smith et al., 2000). Co-grazing of two animal species that are both raised for production purposes, e.g., beef cattle and sheep, is also used in extensive systems. To our knowledge, studies on co-grazed species have largely focused on grazing behaviour of ruminants and reaction to predators. Cattle grazing simultaneously with sheep ruminated and rested longer than those grazing only with conspecifics, whereas sheep covered larger distances than those in single-species grazing systems, which may be due to sheep being pushed from preferred grazing spots by cattle (Cuchillo-Hilario et al., 2017). Sheep sought refuge with cattle during predator attacks and lambs were less stressed at weaning when the flock was forced to associate with cattle by co-grazing the two species in limited space prior to release to extensive pastures (Anderson, 1998; Anderson et al., 2012; Hulet et al., 1989).

Effects of co-grazing on behaviour other than grazing and possible subsequent welfare consequences have to our knowledge not been investigated. If, however, multi-species livestock farming is implemented more widely, it is essential to investigate the effect on behaviour of specific species combinations to ensure the animals' welfare is not compromised and to detect potential positive effects.

While there is limited knowledge on welfare consequences of co-grazing livestock species, the welfare of co-housed zoo species has received more research attention. Co-housing different animal species in zoos and safari parks has been postulated as possibly enriching due to social interactions (Daoudi et al., 2017; Rowden and Rose, 2016; Veasey and Hammer, 2010). A large difference in body size between two species and no or little overlap in feed preferences could be beneficial for successful co-habitation (Veasey and Hammer, 2010). In some instances of successful co-housing a positive effect on behaviour was found, e.g. two primate species in a mixed zoo exhibit displayed a greater willingness to approach a novel object when compared to their single-species housed conspecifics (Hardie and Buchanan-Smith, 2000). Comparative studies in co-habited livestock species are missing to date, but could shed light on possible welfare consequences and should therefore be conducted for different livestock species combinations. While the prevalence of two or more livestock species on one farm and therefore also the prevalence of co-grazing livestock species and species combinations is unknown, an interview study with organic multi-species livestock farmers in seven European countries revealed the combination of cattle and poultry as one of the most prevalent (Ulukan et al., 2021).

To this end, the present study aimed to describe behaviour of cattle and broiler chickens when co-grazing, including interactions between and within species. Moreover, the behaviour of the animals in these multi-species groups was compared to that of conspecifics in single-species groups on the same farm. More specifically, we aimed to address the following research questions: (1) How and how often do young cattle and broiler chickens on pasture interact and how often are

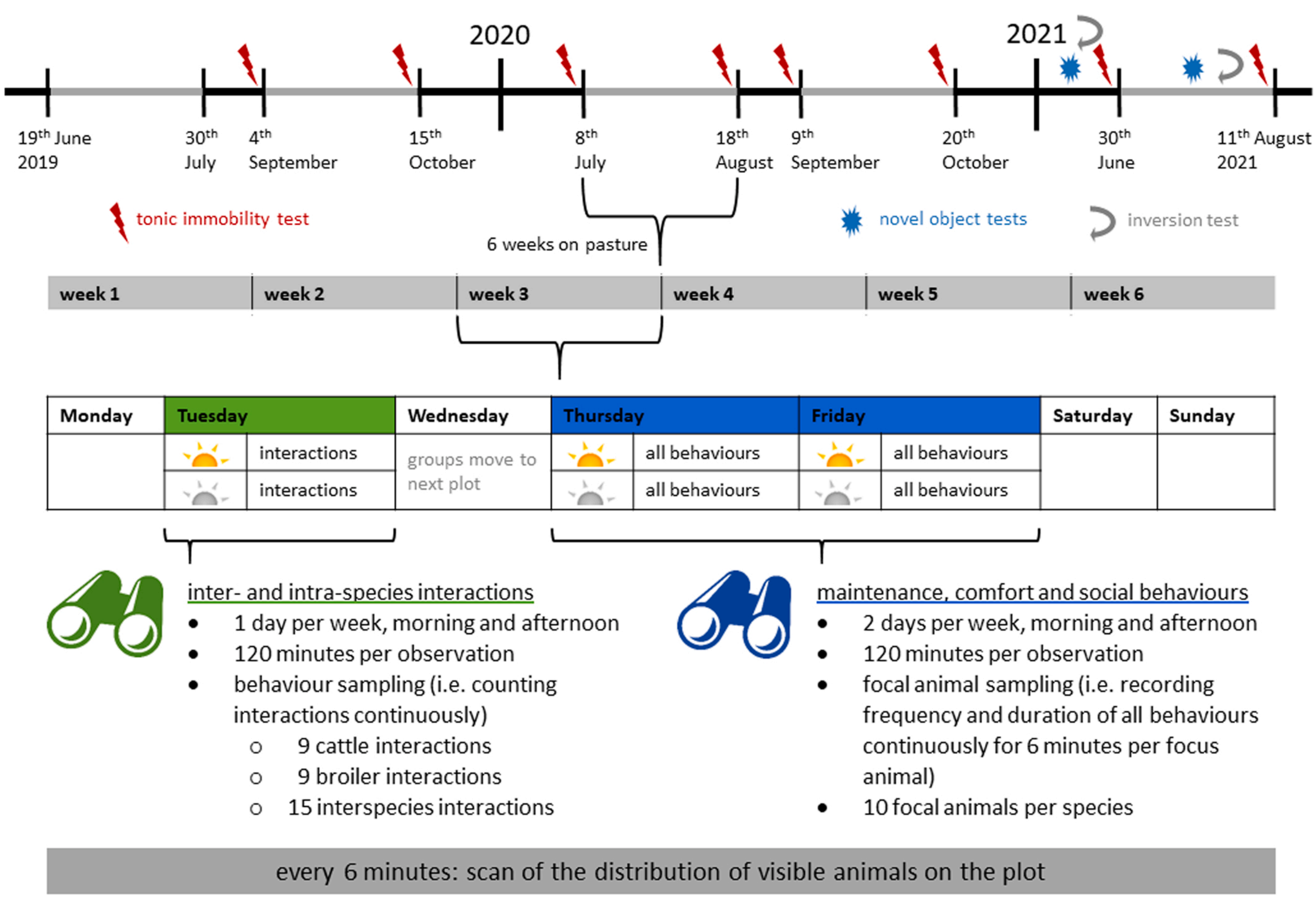

Fig. 1. Timeline and weekly schedule for observations. The five replicates of the six-week cycle are shown in grey with start and finish date in the timeline at the top. Tonic Immobility tests were performed on broilers in the last four cycles (red lightning bolt, research question 3). Novel Object tests (blue explosion) and Inversion tests (grey half-arrow) were only performed with broilers in 2021 to answer additional aspects of research question 3. Behavioural observations were conducted on three days per week. Tuesdays for research question 1 and 2 assessing inter- and intra-species interactions. Thursdays and Fridays frequencies and durations of maintenance, comfort and social behaviours were assessed in non-simultaneous observations for research question 2 (except in 2021 when we observed multi- and single-species groups on Thursdays assessing inter- and intra-species interactions). Both groups were observed each day either morning (yellow sun) and afternoon (grey sun) or one morning and one afternoon of two consecutive days.

they close to each other? (2) Which behaviours, including intra-species interactions, do young cattle and broiler chickens show when on pasture in multi- or single-species groups? (3) Does being kept with young cattle on pasture affect fearfulness in broiler chickens compared to broilers in single-species groups?

2. Animals, material and methods

2.1. Experimental design

The co-grazing experiment with young cattle and broilers was repeated five times. The five six- week cycles were conducted between late spring and autumn in 2019, 2020 and 2021 at the research farm of the Thünen Institute for Organic Farming in Westerau, Germany (for exact dates see Fig. 1). In each cycle three groups of animals were kept on pastures, which were each divided into six plots (Fig. 2, A): One single-species group of 10 cattle and one single-species group of 54–61 broilers were kept on one pasture, always separated by one empty plot, while one multi-species group of 10 cattle and 54–61 broilers was kept on a second pasture (Fig. 2, C). Once per week (Wednesday), all groups were moved to the next plot on their pasture. The two pastures were assigned to the single-species groups or the multi-species group in a balanced order across cycles and seasons. One plot on one pasture had a fenced off area with a water hole and trees which was compensated for by extending the plot's short sides by 5 m.

To achieve similar sward height and to ensure that there was one empty plot between the two single-species groups, the single-species cattle groups' pasture period started two weeks ahead of that of the broiler groups and was thereby on the third plot when the single-species broiler group started the pasture period on the first plot. The plot rotation order was identical across weeks in all cycles.

To answer our research questions, we used different sampling methods and recording rules, including behaviour sampling (i.e., recording the occurrence of inter- and intra-species interactions continuously), focal animal sampling (i.e., recording frequencies and durations for maintenance, comfort and social behaviours of one focal animal for six minutes continuously) and scan sampling (i.e., instantaneously recording the number of cattle and visible broilers per sector every six minutes). Additionally, we used tests to assess fearfulness in broilers. More details about the schedule and the different methods are given in Fig. 1 and the following sections.

2.2. Animals and housing

Broilers (ISA JA 757) were housed in mixed-sex groups in a straw bedded hut (3 × 4.6 m) with perching opportunities, had ad libitum access to water and feed (Eiderkraft – Geflügel-Unimastfutter mixed with wheat), and to pasture during daylight hours. The door (0.8 × 0.5 m) of the hut opened and closed automatically at the beginning and end of civil daylight (dawn phase just before sunrise and dusk phase just after sunset, respectively) using light sensors. The broiler hut was positioned at the border of two plots and therefore was only moved every second week, as the automatic door could be switched to the other side of the hut when the broilers moved to the next plot (Fig. 2, A). Broilers arrived 30 days before the start of the pasture period at the experimental farm as one-day-old chicks. They were raised in an indoor area (3.4 × 6.8 m) with an adjacent concrete covered outdoor area (3.1 × 11.3 m) accessible during the day from the age of 14 days. Thereby, broilers were already used to outdoor access via ramp and door when moved to pasture.

Cattle (Holstein-Friesian) had an average age of 220 days (range: 117–310 days) at the beginning of spring cycles and 284 days (189–383 days) at the beginning of late summer cycles. On pasture they had ad libitum access to water and were fed concentrate (0.5 kg per cattle) once daily. The concentrate was a mixture of oats (70 %), wheat (19 %), peas (10 %) and mineral feed complement (1 %) in 2019 and a mixture of oats (56 %), triticale (32 %), beans (10 %) and mineral feed complement (2 %) in 2020 and 2021. Prior to the start of the experiment, the cattle were

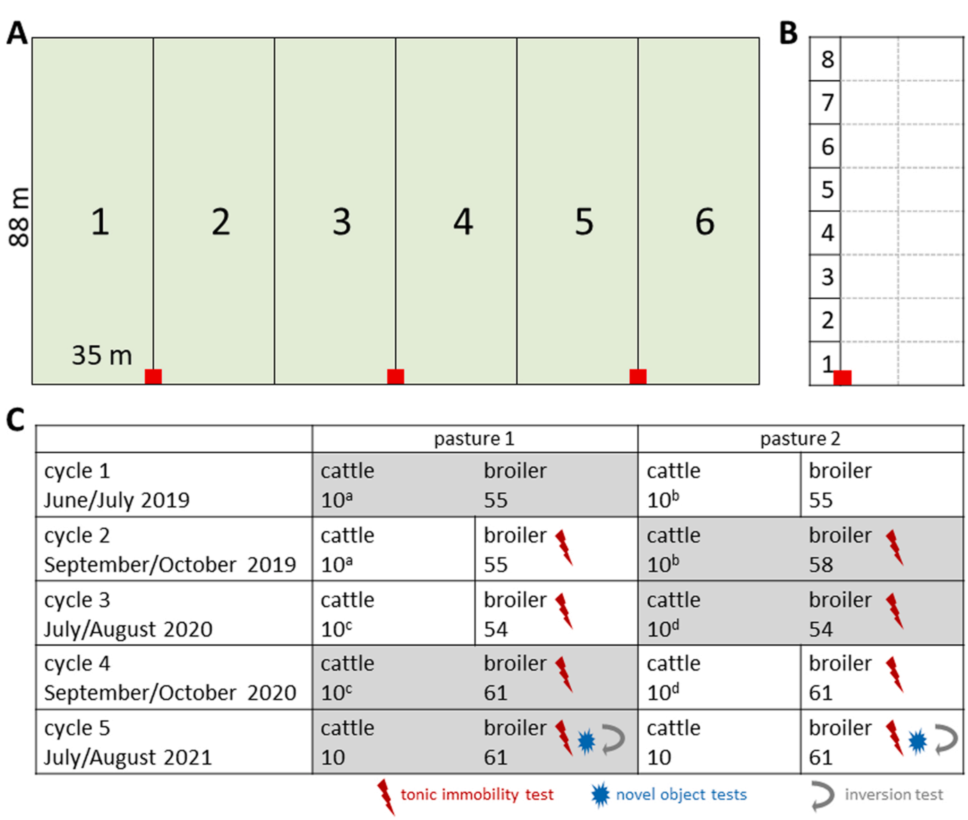

Fig. 2. Schematic layout of a pasture divided into six plots (A), a plot with 16 sectors (B) and the number and distribution of animals across both treatment groups, pastures and cycles (C). (A) Each pasture was separated into 6 plots with the position of the broiler hut marked in red. (B) Each plot was visually subdivided into 16 sectors. (C) Group allocation to pastures 1 and 2 was balanced across the five cycles and seasons. Multi-species groups are marked by grey shading (research question 1), all animals were used to compare multi- and single-species groups (research question 2) and broiler groups marked with symbols (red lightning bolt: Tonic Immobility test, blue explosion: Novel Object test and grey half-arrow: Inversion test) additionally underwent fearfulness tests (research question 3). Cattle groups with the same superscripts were repeatedly used within one year.

Table 1
Ethogram with behaviours recorded in behaviour sampling (b) and focal animal sampling assessed as frequencies or durations (*) with descriptions (and references where available) and partners (for interactions) and their assignment to behaviour categories.

Initiator/ Receiver	behaviour category	behaviour	With whom? (for interactions)	Description
cattle	body position	lying*		Individual touches ground with the torso either laterally or ventrally, body is not supported by the legs (adapted from Winckler et al., 2015).
cattle	body position	standing*		Individual is not lying, supported by its legs and not in motion.
cattle	out of sight	out of sight*		An individual is not visible to the observer.
cattle	consuming	feeding*		Taking food with tongue or teeth in addition to chewing movements.
cattle	consuming	drinking		Lowering mouth into water, while keeping nostrils above and sucking water. Counted as new event after a break of 2 s, during which cattle is not touching water or drinker.
cattle	locomotion	locomotion*		Individual is in motion, not standing. Either walking (four-beat-gait), trotting (two-beat-gait, diagonal pairs moving together, moment of suspension; adapted from Jensen and Kyhn, 2000)
cattle	locomotion	running		Rapid movement with suspension, three- or four-beat-gait. Minimum of 2 consecutive movements of suspension (adapted from Jensen and Kyhn, 2000).
cattle	locomotion	leap/jump/buck		Any lifting of one or both front or hind legs from the ground, while the fore (or back) part of the body is also lifted. Single or repeated upwards or forward movement (adapted from Jensen and Kyhn, 2000). Counted as new event after a 2 s break.
cattle	comfort behaviour	scratching		Scratching itself with one foot or an object, more than two times. Counted as new event after a 2 s break without scratching.
cattle	comfort behaviour	self-grooming		Licking itself anywhere on the body for more than two consecutive licking movements. Counted as new event, after a 2 s break without self-grooming.
cattle	fighting	fighting (b)	cattle	Two individuals are standing front to front, pushing heads against each other. Both are braced (hind legs are behind vertical/normal position and front legs are in front of vertical/ normal position). Ends by one cattle submitting to the other and withdrawing. Often followed by displacement incident (adapted from Winckler et al., 2015).
cattle	mock fighting	horning/frontal pushing/ mock fighting (b)	cattle	Rubbing the forehead and (former) horn buds against the body of another individual in a playful manner (adapted from Duve et al., 2012; Jensen and Kyhn, 2000; Valníčková et al., 2015). Minimum 3 consecutive rubbing movements. Counted as a new event after 2 s break, without contact to other cattle. Neither cattle are braced for pushing.
cattle	mounting	mounting (b)	cattle	Both forelegs lifted off the ground while jumping on the back, side or head of another individual (adapted from Jensen and Kyhn, 2000). Counted as new event after 2 s break.
cattle	object play	object play		Butting water bowl, hayrack, fence or other object with force, but in a playful manner (adapted from Duve et al., 2012; Jensen and Kyhn, 2000). Minimum for three consecutive movements. Counted as new event after 2s break with no contact to object.
cattle	ruminating	ruminating*		Repetitive, uniform chewing movement independent from feed ingestion.
cattle	socio-negative, active interaction	head push (b)	broiler	Cattle is grazing and pushing broiler away with head, with contact (with less than 4 steps moved before). Counted as new event after 2 s break.
cattle	socio-negative, active interaction	displacement conducted (b)	cattle or broiler	Cattle moves towards (more than 4 steps) towards target animal at a swift pace, focusing on the target and oversteps the 1-metre perimeter of the target animal. If target animal leaves with or without physical contact by acting cattle in less than 3 s a displacement was conducted. The target animal has to be displaced by at least one breadth or half a body length for cattle and at least two body length for broilers.
cattle	socio-negative, active interaction	chasing (b)	cattle or broiler	Cattle is following another individual at faster speed and displacing it for at least 5 consecutive seconds, with an aggressive manner, e.g. lowering its head. Counted as new event after a 2 s break.
cattle	socio-negative, active interaction	chasing up (b)	cattle or broiler	One cattle approaches a lying one with purpose and chases it up from its lying position with or without body contact.
cattle	socio-negative, passive interaction	displacement received (b)	cattle or broiler	Cattle is being targeted and displaced from grazing site by another individual through overstepping 1 m perimeter and leaving with or without body contact by at least one breadth or half a body length for cattle and at least two body length for broilers.
cattle	other active interaction	licking (b)	cattle	One individual performs tongue movements on another's body or head (excluding ano-genital region), more than two consecutive movements (adapted from Winckler et al., 2015). Counted as new event after a 2 s break.
cattle	other active interaction	proximity with muzzle (b)	cattle or broiler	Individual (almost) touches head or body with the muzzle, in close proximity or in contact (~10 cm) of the skin. Counted as new event after a 2 s break.
cattle	other active interaction	approaching (b)	cattle or broiler	Cattle moves towards (more than 4 steps) towards target animal at a swift pace, focusing on the target oversteps the 1-metre perimeter of the target animal. If target animal remains for at least 3 s it was approached and tolerates the proximity. Counted as new behaviour when 1-metre perimeter had been left and is overstepped again.
cattle	other active interaction	following (b)	cattle or broiler	Cattle is following behind or at the side of another individual at similar speed. Counted as new event after a 2 s break.
cattle	other passive interaction	being approached/ tolerating proximity (b)	cattle or broiler	Cattle is being targeted and approached by another individual by overstepping 1-metre radius and remaining or not changing direction or speed for at least 3 s. Counting as new event when 1-metre perimeter had been left and is overstepped again.
broiler	body position	lying*		Sitting with both legs bent and abdomen in contact with ground or on side; including all neck positions (adapted from Wallenbeck et al., 2016).
broiler	body position	standing*		Individual is not lying, supported by its legs and not in motion, no other body part is touching ground (Wallenbeck et al., 2016).
broiler	out of sight	out of sight*		An individual is not visible to the observer.
broiler	locomotion	locomotion*		Walking and running (including running steps with both feet in the air) for 2 s or more (Wallenbeck et al., 2016).
broiler	locomotion	group running* (b)		

(*continued on next page*)

Table 1 (*continued*)

Initiator/ Receiver	behaviour category	behaviour	With whom? (for interactions)	Description
				Three or more broilers are running together, close to each other and in same direction (Wallenbeck et al., 2016). Running must stop in front of house/ramp. Counted as new event after 2 s break.
broiler	explorative behaviour	pecking at ground/ foraging*		Consuming or manipulating vegetal substrate (adapted from Wallenbeck et al., 2016).
broiler	explorative behaviour	scratching the ground		Broiler explores ground with feet (Gonçalves et al., 2017). Counted as new event after 2 s break.
broiler	explorative behaviour	scratching/ pecking at dung pad		Broiler explores dung pad with feet or beak. Counted as new event after 2 s break.
broiler	comfort behaviour	preening*		Exploring the feathering with beak for e.g. maintenance (adapted from Gonçalves et al., 2017; Wallenbeck et al., 2016). Counted as new event after 2 s break.
broiler	comfort behaviour	dust bathing		Wingshake/ fluffed feathers in sitting/lying position, followed by side or head rubs lying on the side, motion of the legs possible, scratching at ground; pecking at ground (adapted from Wallenbeck et al., 2016). Counted as new event after 2 s break.
broiler	comfort behaviour	wing or leg stretch		Slowly stretching out one wing and/or one leg (Wallenbeck et al., 2016). Counted as new event after 2 s break.
broiler	play fighting	play fighting* (b)	Other broiler	Two broilers pushing their feet at each other at least once, often coinciding with running; also includes standing in front of each other and pecking at each other (for at least two times, adapted from Wallenbeck et al., 2016). Running at each other, stopping and remaining motionless, staring at each other for at least 2 s.
broiler	wing flapping	wing flapping		Stretching out and flapping wings once or more times (Wallenbeck et al., 2016). Counted as new event after 2 s break.
broiler	seeking shelter	seeking shelter with cattle (b)	cattle	Seeks shelter/proximity (overstepping 1-metre perimeter) to cattle due to predatory bird or plane or another object.
broiler	seeking shelter	seeking shelter in house (b)		More than two broilers seek shelter in house due to predatory bird or plane or another object. At least 50 % of running group must enter house.
broiler	sexual behaviour	mating	broiler	One broiler sits on top of another broiler. Counted as new event after 2 s break.
broiler	socio-negative, active interactions	displacement conducted (b)	cattle or broiler	Broiler shows directional/purposeful locomotor behaviour towards target animal and oversteps the 1-metre perimeter of the target animal. If target animal leaves a displacement was conducted. The target animal has to be displaced by at least one breadth or half a body length for cattle and at least two body length for broilers.
broiler	socio-negative, active interactions	chasing up (b)	cattle or broiler	One broiler approaches a lying one with purpose and chases it up from its lying position with or without body contact.
broiler	socio-negative, active interactions	pecking at other individual (b)	cattle or broiler	Pecking at feathers/skin of another broiler/cattle (adapted from Wallenbeck et al., 2016). Counted as new event after 2 s break.
broiler	socio-negative, passive interactions	displacement received (b)	cattle or broiler	Broiler is being targeted and displaced from grazing site etc. by another individual through overstepping 1 m radius and leaving with or without body contact by at least one breadth or half a body length for cattle and at least two body lengths for broilers.
broiler	other active interactions	approaching (b)	cattle or broiler	Broiler is approaching other broiler/cattle and both are staying in proximity for at least 3 s. Counting as new event when 1-metre perimeter had been left and is overstepped again.
broiler	other active interactions	proximity with peak (b)	cattle or broiler	Beak is in close proximity to any part of the body of the other individual. Counted as new event after 2 s break.
broiler	other active interactions	following (b)	cattle or broiler	Broiler is following behind or at the side of another individual at similar speed. Counted as new event after a 2 s break.
broiler	other passive interactions	being approached/ tolerating proximity (b)	cattle or broiler	Broiler is being targeted and approached by another individual through overstepping 1-metre radius and remaining for at least 3 s. Counting as new event when 1-metre perimeter had been left and is overstepped again.

housed indoors but had several days of previous experience with pasture before the start of the experiment.

Within each cycle the same ten cattle in one group were observed across weeks. In each calendar year, cattle from the first cycle switched from single-species group to multi-species group and vice versa for the second cycle (see animal superscripts in Fig. 2, C), resembling a split-plot design with cattle in one calendar year being used in both cycles (switching treatments in the second cycle).

2.3. Data collection and processing

2.3.1. Behavioural observations in multi- and single-species groups

All behavioural observations lasted for 120 min and started in the morning shortly after the beginning of civil daylight (dawn phase just before sunrise) or ended in the evening shortly before the end of civil daylight (dusk phase just after sunset), as previous research found increased activity in broilers during these periods (e.g. Savory, 1976; Yeates, 1963). The observation schedule was identical across weeks in all cycles, with the exception of observations for the frequency and duration of behaviours in 2021, which were replaced by observations assessing only intra-species interactions in single-species groups to increase the sample size for research question 2 comparing intra-species interactions between single- and multi-species groups. Observations were mainly conducted by LS and SHue.

We observed from a high seat positioned at the plot's short side closest to the broiler hut, approximately six metres from the fence line and approximately eight metres from the extension of the long fence line opposite the broiler hut (marked in red in Fig. 2, A and B).

2.3.1.1. Proximity and distribution of visible animals (relevant for research questions 1 and 2). To assess proximity between species, all observations were halted every 6 min (instantaneous scan sampling) to record the position of all cattle and visible broilers (i.e. not in hut) within the plot (research question 1). To record positions, each plot was divided into 16 sectors by visually splitting it once along its short side and into eight equal sections along its long side (Fig. 2, B). For ease of recording the location of animals, we placed numbers 1–8 along both long fence lines and white rubber tubes with a small diameter along the centre of the plot, parallel to the long sides. All scans were done on paper and later transferred to Microsoft Excel files (Excel 2016, MSO 16.0.4266.1001).

Based on all scans we calculated the average number of broilers outside the hut in multi-species groups (n = 2331 scans, 111 observations × 21 scans) and single-species groups (n = 1785 scans, 85 observations × 21 scans). Proximity between species in multi-species groups was assessed by calculating the number of sectors with cattle or broilers and the number of sectors with both species. The number of sectors with both species was divided by the number of sectors with either cattle or broilers resulting in a percentage of scans in which broilers were observed in sectors with cattle and cattle were observed in sectors with broilers.

2.3.1.2. Inter-species interactions (research question 1). Multi-species groups were observed in the morning and evening of one day per week (Tuesday) using behaviour sampling, i.e. counting the occurrence of pre-selected inter-species interactions, for 120 min each (see Table 1 and for selected illustrations Fig. 3). Inter-species interactions were described based on pilot observations due to a lack of information from the literature. Behaviour occurrences were recorded on paper and later transferred to Microsoft Excel files (Excel 2016, MSO 16.0.4266.1001). The multi-species groups were observed for in total 118 h (59 × 120 min).

2.3.1.3. Frequency and duration of maintenance, comfort and social behaviours including intra-species interactions (research question 2). To quantify intra-species interactions in multi- and single-species groups, multi- and single-species groups were observed in the morning and evening of one (Tuesday) or two days per week (in 2021) using continuous behaviour sampling for 120 min each. Behaviour definitions from previously published ethograms were used and where necessary expanded. In total, we observed the broiler single-species groups in cycles 2–5 for 76 h (38 × 120 min). The number of observations for the single-species cattle groups was with 14 h (7 × 120 min) considerably lower than for the single-species broiler groups as they were only observed in cycles 4 and 5 and the single-species cattle groups finished their six-week pasture period two weeks earlier than the other two groups.

Using behaviour definitions from previously published ethograms, a wide range of behaviours (maintenance, comfort and social behaviour; see Table 1) was additionally recorded on two days either in the morning or the evening using continuous focal animal sampling of all ten cattle and ten randomly chosen broilers for six minutes each (Thursday/Friday). The focal animal species was switched after each individual and the species with which the observation began was alternated. Focal broilers were randomly selected by visually splitting the area occupied by broilers outside the hut, at the time of selection, into three sections of equal size with varying distances from the hut and choosing broilers from each area in a balanced order. If the focal broiler disappeared into the hut, 'out of sight' was recorded, the broiler nearest to the door of the hut was selected and its behaviour continuously recorded for the remainder of the six-minute period. Since broilers were similar in appearance and not visibly marked as individuals, it cannot be excluded that one broiler was observed repeatedly. For cattle the order of observation was chosen randomly prior to observations and individual cattle were identified by coloured neck collars. Observations were scheduled in a balanced order accounting for time of day across days and weeks.

The behaviour of the focal animal was recorded using Mangold Interact Software (18.5.1.0). We recorded the frequency of maintenance, comfort and social behaviours as well as the time spent performing maintenance behaviours (marked with * in Table 1), e.g., frequency of lying events as well as time spent lying (later expressed as proportion of time). Behaviours were summarised according to behaviour categories (Table 1).

Both the multi-species groups and the broiler single-species groups were observed for 46 h using continuous focal animal sampling (46 observations x 10 animals x 6 min), whereas the cattle single-species group was observed for 29 h (29 observations × 10 cattle × 6 min).

2.3.2. Fearfulness in broiler chickens as measured by Tonic Immobility (TI), Inversion and Novel Object tests (research question 3)

As co-grazing with cattle could be enriching for broilers, we expected broilers in multi-species groups to be less fearful than those in single-species groups. We tested fearfulness in broiler chickens using a Tonic Immobility test, an Inversion test and a Novel Object test. Tonic Immobility (TI) tests were performed with broilers in all cycles except the first one in 2019 (Fig. 2, C). The first TI test per cycle was done one or two days prior to the start of the pasture period at four weeks of age and the second test was done one or two days prior to the end of the pasture period at the age of ten weeks.

Each broiler was caught individually from within their enclosure/hut and carried in both hands for approximately five metres to the testing area. For the test at the age of four weeks a room adjacent to the raising pen and for the test at the age of ten weeks a trailer with closed side walls and open roof was used. In the testing area each broiler was placed on its back in a v-shaped cradle and held with one hand over the sternum and the other over the head for 10 s (Benoff and Siegel, 1976; Forkman et al., 2007; Jones and Faure, 1981; Zulkifli et al., 2000). Upon releasing the broiler, the experimenter stepped back by approximately one metre, averted her gaze and refrained from eye contact with the broiler or any unnecessary movements or noises. If TI was induced (i.e., broiler remained lying for at least 10 s) the time until first alert head movement (i.e., scanning movements) and until self-righting was measured (maximum 600 s) (Jones, 1986). When a broiler did not right itself within this timeframe, the experimenter helped the broiler up before returning it to its conspecifics. If TI was not induced at the first attempt the procedure was repeated for a maximum of five times. The number of attempts necessary to induce TI and the duration spent in TI was recorded using a stopwatch and a Microsoft Excel table. Based on the results of the first test (at 4 weeks of age) broilers were ranked according to their time spent in TI and every second broiler was assigned to the single- or multi-species group (Wang et al., 2013), thereby creating two groups with similar means for the duration spent in TI. The group assignment for some randomly chosen broilers were switched to end up with two groups with identical means for the duration spent in TI. All TI tests were additionally recorded with a video camera (Panasonic HX-WA30). Number of attempts necessary to induce TI, latency to first alert head movement (s) and duration in TI (s) were assessed for all TI tests by two observers using the video material. Results are compared between single and multi-species groups for the second TI test.

For the cycle in 2021, we additionally performed an Inversion test, which measures the initial fear reaction to a simulated attack and is said to be correlated with the TI test (Newberry and Blair, 1993), with a random subsample of 40 broilers (i.e., 20 per group) at two weeks of age and again at 8 weeks of age, i.e. two weeks before each TI test, to reduce the possibility of cross-influencing results. The broilers were individually caught and carried to the testing arena in which they were held with one hand by their feet and turned upside down until wing flapping ceased or for a maximum of 30 s (Archer and Mench, 2014). They were then righted and returned to their enclosure. All Inversion tests were recorded using a video camera (Panasonic HX-WA30). Data were extracted from video recordings watched in slow-motion. The intensity of the reaction was calculated by dividing the number of wingbeats by the time wing flapping was performed (Archer and Mench, 2014).

Additionally, with broilers from the cycle in 2021, three Novel Object tests were performed, one at three, one at four and one at nine weeks of age. For the first two tests, i.e. before the start of the experiment, all broilers were tested in the same group and for the third test all broilers in single- and multi-species groups were tested in their respective groups. For each test a camera (GoPro Hero 7) was installed at least 30 min before the beginning of the test in the home pen directly above of the location where the novel object would be placed. The experimenter then placed the novel object in the pen and left. Broilers were first tested with a PVC pipe (30 cm in length) wrapped in yellow, red and blue tape, for the second test we used a traffic cone lying on its side and for the third an inflatable ball with differently coloured figures on it. We assessed the latency until the first three broilers crossed a 25 cm radius around the novel object and the number of broilers within this radius every 10 s for 120 s from the video footage (De Haas et al., 2014; Welfare Quality®, 2009).

2.4. Assessment of inter-observer agreement

We assessed inter-observer agreement between two observers for the distribution of visible animals, inter- and intra-species interactions, the frequencies and durations of maintenance, comfort and social behaviours as well as for the Tonic Immobility, Inversion and Novel Object tests.

For the distribution of animals ($n = 367$ scans) the recorded number of broilers visible and the number of animals in each sector (Fig. 2, B) for cattle and broilers were compared. For inter- and intra-species interactions ($n = 5$ observations, 120 min each) all interactions which were coded at least once by each observer and the total number of interactions recorded were compared. For the frequencies and durations ($n = 150$ cattle observations, $n = 190$ broiler observations, 6 min each) all behaviours that were recorded at least twice were compared for maintenance, comfort and social behaviours. For the TI test ($n = 33$ broilers) number of attempts necessary to induce TI, latency to first alert head movement (s) and duration in TI (s) and were compared. For the Inversion test ($n = 9$ broilers) we compared time inversed (s) and number of wingbeats and for the Novel Object test ($n = 48$, 12 time points per test) the number of broilers close to the novel object.

As a measure of inter-observer agreement, the Intraclass Correlation Coefficient (ICC) estimates and their 95 % confidence intervals (CI) were calculated based on single measurements, absolute agreement and a 2-way (instead of 1-way, since there is no 'correct' observer) mixed effects model for all inter-observer agreement calculations. ICC values between 0.5 and 0.75 indicate moderate agreement, values between 0.75 and 0.9 indicate good agreement and values above 0.9 indicate excellent agreement (Koo and Li, 2016); additionally, the lower CI should be within the lower limit of the agreement categories.

2.5. Robustness of data collected from behavioural observations

Since single- and multi-species groups were observed in a balanced order considering time of day across weeks (i.e., non-simultaneously), conditions during observation sessions (i.e. mornings vs. evenings, or during consecutive days) such as aspects of the weather could have affected the occurrence of behaviours. Although we assumed the magnitude of such effects to be small due to the large number of observation periods, to further assess the robustness of our data LS and SHue observed single- and multi-species groups simultaneously during 25 sessions ($n = 50$ h). If occurrences were in a similar range for both simultaneous and non-simultaneous observations, it could indicate that the specific conditions did not systematically affect occurrences of behaviour in non-simultaneous observations.

Frequency of behaviours and proportion of time spent performing maintenance, comfort and social behaviours for single- and multi-species groups obtained from simultaneous observations were within a similar range as the values obtained from the non-simultaneous observations. Similar to non-simultaneous observations, simultaneous observations revealed only slight differences in medians and Inter-Quartile-Range (IQR) for frequencies of behaviours and proportions of time between multi- and single-species groups (for detailed information see Supplementary material Table S4).

2.6. Descriptive analyses

Due to the small sample size ($n = 5$ replicates/cycles for observations of inter- and intra-species interactions, $n = 4$ cycles for maintenance, comfort and social behaviours and the TI test, and $n = 1$ for Novel Object and Inversion tests), we only performed descriptive statistics and visual inspection of the data using the statistical programming language R (R Version: 4.0.3, R Core Team, 2020; RStudio Version: 1.4.1103, RStudioTeam, 2021), package 'irr' to calculate the Intra Class Correlation Coefficients (Gamer et al., 2012) and package 'ggplot2' to create graphs (Wickham, 2016).

Inter-species interactions were calculated per hour and 10 animals per species and intra-species interactions per hour and 10 cattle or broilers. Frequencies of behaviours were calculated per hour and animal (reported as median and Inter-Quartile-Range IQR due to the zero inflated distribution) and durations as proportion of time (reported as mean and standard deviation (sd)).

For the Inversion test the difference (delta) between values obtained before and after the pasture period was calculated and compared between groups. To assess a possible association between duration in TI and intensity after pasture, a Spearman's rank correlation coefficient was calculated.

2.7. Ethical considerations

The ethical guidelines of the International Society of Applied Ethology were followed during this experiment. This experiment was part of a larger research project and all procedures were approved by the Ministry of Energy, Agriculture, the Environment, Nature and Digitalisation in Schleswig-Holstein (reference V242–46376/2019; V242–26697/2021).

3. Results

3.1. Inter-observer agreement

For the distribution of visible animals, the agreement between observers was moderate to excellent for the number of cattle in all but one sector and for the number of broilers in the three sectors in which most broilers were observed (for detailed information see Supplementary material Table S2). For the total number of broilers visible, the agreement was excellent (ICC, lower CI < ICC < upper CI: 0.931, $0.916 < ICC < 0.944$, $F(356, 353) = 28.2$, $p < 0.001$).

The agreement between observers for inter- and intra-species interactions could, due to the low number of occurrences for some behaviours, only be calculated for 13 out of 19 cattle-initiated behaviours and 7 out of 15 broiler-initiated behaviours for which sufficient data were available (for detailed information see Supplementary material, Table S1). Observer agreement was moderate to excellent for nine cattle-initiated behaviours (Fig. 4, A) and five broiler-initiated behaviours (Fig. 4, B) and poor for four cattle-initiated behaviours and two broiler-initiated behaviours.

Agreement between observers for frequencies and durations of maintenance, comfort and social behaviours was (almost) excellent ($ICC > 0.75$) for 16 cattle behaviours (Fig. 4, A) and 14 broiler behaviours (Fig. 4, B; for more detailed information see Supplementary material, Table S1) and moderate for one cattle behaviour and three broiler behaviours (ICC and lower $CI > 0.5$). Five cattle and two broiler behaviours were not recorded during these observation sessions.

For the fearfulness measures, agreement was excellent for duration spent in TI in seconds (0.999, $0.999 < ICC < 1$, $F(32, 33) = 2945$), latency to first alert head movement in seconds (0.934, $0.87 < ICC < 0.967$, $F(33, 32) = 28.3$) and number of attempts necessary to induce TI (0.989, $0.978 < ICC < 0.994$, $F(32,33) = 180$). Agreement was also excellent for time inversed (0.988, $0.951 < ICC < 0.997$, $F(8, 8.35) = 192$) and number of broilers near the novel object (0.978, $0.959 < ICC < 0.988$, $F(47, 40.1) = 94.3$) (all p-values $p < 0.001$). For number of wingbeats the lower bound CI was below the level for moderate agreement (0.969, $0.312 < ICC < 0.995$, $F(8, 1.83) = 212$, $p < 0.01$).

3.2. Inter-species interactions in multi-species groups (research question 1)

On average 13 ± 9 broilers were visible (i.e., not in hut) in multi-species groups. When cattle were observed in a sector, in approximately 10 % of cases were broilers also observed in the same sector, while in approximately 39 % of cases cattle were observed when broilers were present in a sector.

The number of inter-species interactions recorded varied between observation periods and cycles and totalled in 787 inter-species across 118 h (59 observations, 120 min each). With an average of 13 visible broilers in multi-species groups and a mean of 7 inter-species interactions in one hour approximately one inter-species interaction per visible broiler and ten cattle occurs in two hours. The most frequent interactions were cattle displacing broiler and broiler approaching cattle (for details on the occurrence of individual inter-species interactions see Fig. 5, A and Supplementary material Table S3). Inter-species interactions occurred similarly often in the morning (5 ± 7) and evening (5 ± 5).

3.3. Intra-species interactions of young cattle and broiler chickens in multi- and single-species groups (research question 2)

On average 8 ± 6 broilers were visible (i.e., not in hut) in single-species groups. Based on behaviour sampling, both cattle and visible broilers interacted with a conspecific on average twice per hour and animal in multi-species groups. Intra-species interactions in the single-species cattle groups occurred at a similar frequency, but broiler-broiler interactions in the single-species broiler groups amounted to on average three interactions per hour (details on intra-species interactions can be found in Fig. 6, A for broilers and Fig. 6, C for cattle and in the Supplementary material Table S3). Most intra-species interactions were performed similarly often in both single- and multi-species groups, with the exception of broilers in single-species groups performing numerically more 'other social behaviour' (e.g., approaching and following; mean ± sd: 15 ± 7 per hour and 10 animals) than broilers in multi-species groups (9 ± 12). The variability between observations was high in both groups.

Similarly, focal animal observations revealed no noteworthy differences in the average number of intra-species interactions for both cattle and broilers in multi- and single-species groups (Fig. 6, E for broilers and Fig. 6, F for cattle and Supplementary material Table S4). Intra-species interactions between broilers (calculated per 10 broilers and hour) occurred numerically more often in the morning (mean ± sd: multi-species groups 29 ± 32, single-species groups 32 ± 17) than in the evening (multi-species groups 16 ± 17, single-species groups 25 ± 10) and between cattle (calculated per 10 cattle and hour) numerically slightly more often in the evening (mean ± sd: multi-species groups 26 ± 16, single-species groups 20 ± 7) than in the morning (multi-species groups 18 ± 13, single-species groups 16 ± 5).

3.4. Frequency of and proportion of time spent performing maintenance, comfort and social behaviours in multi- and single-species groups (research question 2)

Variation in the frequency and duration of maintenance, comfort and social behaviours was large in both multi- and single-species groups. There were only minor numerical differences between the frequency of (broiler: Fig. 6, E; cattle: Fig. 6, F) and proportion of time spent performing maintenance, comfort and social behaviours (broiler: Fig. 6, B; cattle: Fig. 6, D) in multi- and single-species groups (for further details see Supplementary material Table S4). The numerically highest difference between groups was for the frequency of a broiler entering the hut ('out of sight', median, IQR: multi-species groups 5, 6 vs. single-species group 9, 7) and the proportion of time all broilers were in the hut (i.e. no broiler visible, mean ± sd: multi 16 % ± 27 vs. single 25 % ± 24).

For behaviours expressed as proportions of time other notable numerical differences were for cattle lying (mean ± sd: multi 21 % ± 20 vs. single 27 % ± 21) and standing (9 % ± 7 vs. 6 % ± 5)). For detailed information see Supplementary material Table S4.

3.5. Fearfulness measures in broiler chickens in multi- and single-species groups as measured by Tonic Immobility (TI), Inversion and Novel Object test (research question 3)

At the end of the pasture period broilers co-grazing with cattle did not differ numerically for TI duration (mean ± sd: 131 s ± 146) from broilers ranging only with conspecifics (126 s ± 137, Fig. 7, A). Likewise, no differences were found for the number of attempts necessary to induce TI (2 ± 1 for both groups, Fig. 7, C) or for the latency to first alert head movement (multi-species group: 12 s ± 27, single-species group: 14 s ± 39, Fig. 7, B).

The number of wingbeats per second in the Inversion test after pasture was 8 ± 1 for both groups and before pasture 9 ± 1 for broilers subsequently assigned to the single-species broiler group and 9 ± 2 for broilers assigned to co-graze with cattle. The difference between before and after pasture was 1.6 ± 1.9 for broilers ranging only with conspecifics and 0.8 ± 1.8 for broilers co-grazing with cattle (Fig. 7, D). The duration in TI after pasture was only weakly correlated with the measure of intensity of reaction to inversion (i.e. wingbeats per second) in the Inversion test after pasture ($rho = 0.3$, $p = 0.7$).

The latency to approach was shorter and the number of broilers approaching a novel object was numerically higher for broilers tested before pasture (all in one group: PVC pipe and traffic cone) than both groups towards the end of the pasture period (inflatable ball). The latency for the first three broilers to cross the 25 cm radius around the novel object was 22 s and 27 s for the PVC pipe and the traffic cone, respectively, and for the inflatable ball 106 s and more than 120 s for the single- and multi-species group, respectively. The number of broilers within a 25 cm radius around the novel object was at every observed timepoint numerically higher before pasture than after pasture, but no notable difference between multi- and single-species groups towards the end of the pasture period was found (Fig. 7, E). For example, at 100 s there were more than 12 broilers within the radius in both tests before pasture and only two (single-species) or three (multi-species) broilers in the one test after pasture.

4. Discussion

Our study aimed to investigate behaviour of young cattle and broiler chickens on pasture in single- and multi-species groups using different observation methods and fearfulness tests. Inter-species interactions occurred approximately two to three times per hour and ten animals per species, with cattle displacing broiler and broiler approaching cattle being most frequent. It was more likely to observe cattle in a sector in which broilers were observed than broilers in a sector with cattle (research question 1). Broilers in single-species groups interacted more frequently with conspecifics than broilers in multi-species groups. Otherwise, we did not find a noteworthy numerical difference between single- and multi-species groups for most behaviours (research question 2) including measures of fearfulness (research question 3).

We were able to reliably assess the majority of recorded behaviours. Those behaviours with a low inter-observer agreement occurred rarely during the time the two observers collected data for agreement. For behaviours occurring less frequently, missing one event by one of the observers has a higher impact on agreement than for more prevalent behaviours. More generally, the definitions of behaviour with low agreement could possibly be improved or observers could be trained more thoroughly prior to the start of the data collection using video material of rarely occurring behaviours, thereby increasing agreement. To further determine the robustness of our data collection setup we showed that the results from non-simultaneous and simultaneous

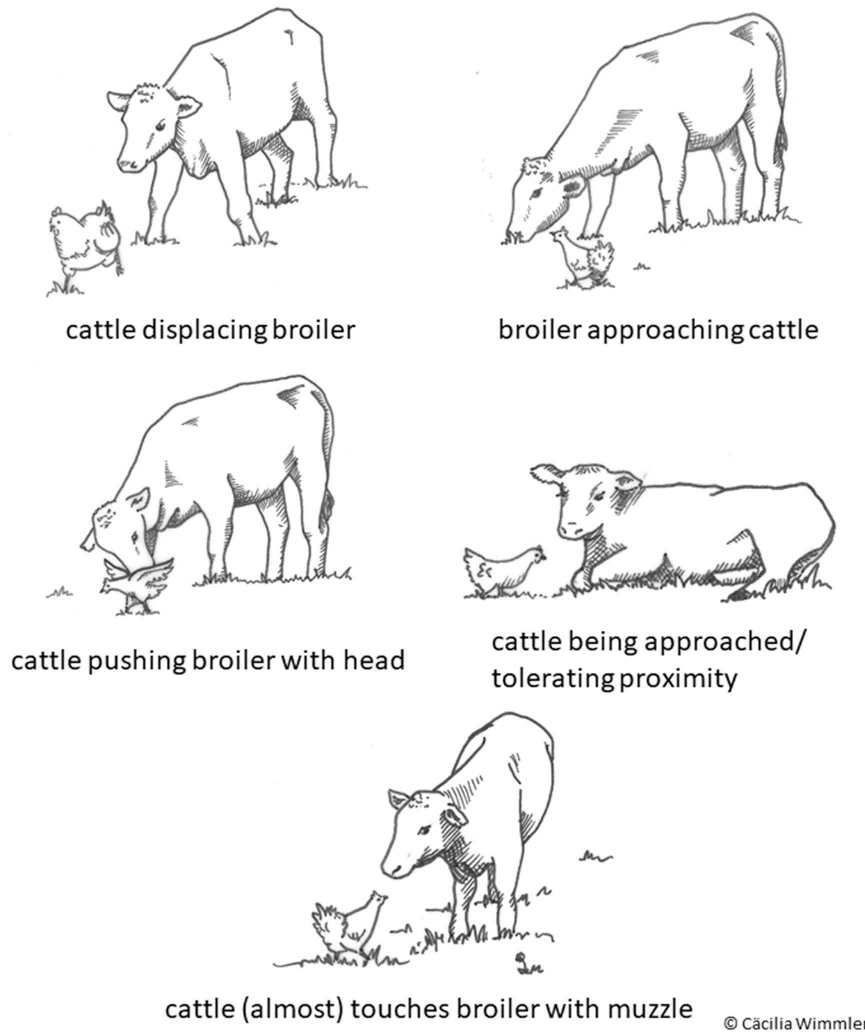

Fig. 3. Illustrations of selected interactions between cattle and broilers.

observations hardly differed. Therefore, we can conclude that observation-specific aspects such as weather did not affect behaviour in non-simultaneous observations in our study, which could be due to such aspects not affecting behaviour or, more likely, due to a sufficiently large sample size reducing the effect of individual observations.

Both presumably negative (i.e., displacing) and positive (i.e. approaching) inter-species interactions occurred in multi-species groups and were initiated by both cattle and broilers. Broilers approached cattle (once per hour and 10 animals per species) despite the relatively frequent experience of being displaced by cattle (two to three times per hour and 10 animals per species), which could be interpreted as broilers not perceiving displacement by cattle as threatening or frightening. However, as we do not know whether the same broilers who were displaced by cattle were the broilers approaching cattle, we cannot be certain how broilers generally perceive interactions with cattle and future studies could mark each broiler uniquely to answer this question. From the cattle perspective, frequent displacements of broilers could be interpreted as cattle not wanting to be in close proximity to broilers. However, since the pasture had sections where broilers rarely ventured, cattle would have been able to avoid proximity by avoiding the sectors close to the broiler hut and broilers would have been able to avoid cattle by entering the broiler hut. Overall, inter-species interactions were initiated by both species, indicating that the animals were interested in the interaction with the other species.

The high percentage of scans with cattle being observed in broiler sectors (39 %) could be due to broilers occupying mainly sectors close to the hut, thereby avoiding traversing large distances across open spaces (Dawkins et al., 2003), while cattle were using the whole plot. However, the presence of cattle apparently did not deter broilers from venturing outside the hut, thus supporting the interpretation that staying in the same sector of the pasture was not perceived as frightening by the broilers. On the contrary, on average more broilers in multi-species groups were outside their hut (average number: 13) than broilers in single-species groups (8). This may indicate that broilers perceived cattle as a structural element on the otherwise barren pasture (Fanatico et al., 2016), and therefore encouraged them to be outside in greater numbers.

A second noteworthy difference in broiler behaviour between multi- and single-species groups was that broilers in multi-species groups interacted less frequently with other broilers compared to broilers in single-species groups, more specifically they approached and followed each other less frequently. This may suggest that broilers in multi-species groups had the opportunity to interact with cattle and performed such interactions with cattle in a similar magnitude as the difference in the number of intra-species interactions between groups. Apart from these differences, co-grazing with another livestock species did not affect cattle or broiler behaviour. A wide range of behaviours, including maintenance behaviours such as lying and feeding, comfort behaviours such as self-grooming or preening, and social behaviours such as play fighting, occurred similarly frequent for cattle and broilers in both multi- and single-species groups, but with a high variation between cycles and observations. Inter-species interactions in the multi-

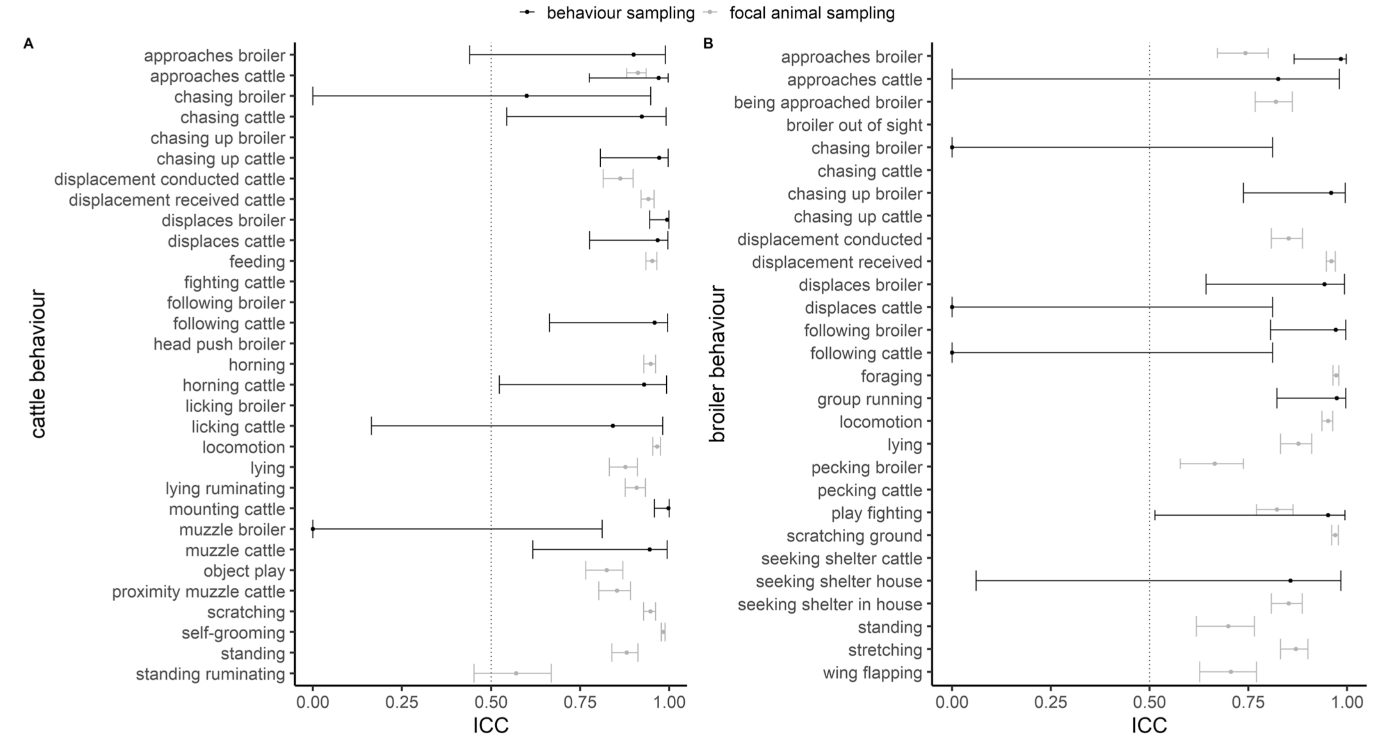

Fig. 4. Inter-observer agreement between two observers calculated with the Interclass Correlation Coefficient (ICC, dot) with Confidence Intervals (CI, whiskers) for cattle (A) and broiler behaviours (B) and the methods behaviour sampling (inter- and intra-species interactions, black) and focal animal sampling (behaviours including maintenance, comfort and social behaviours, grey).

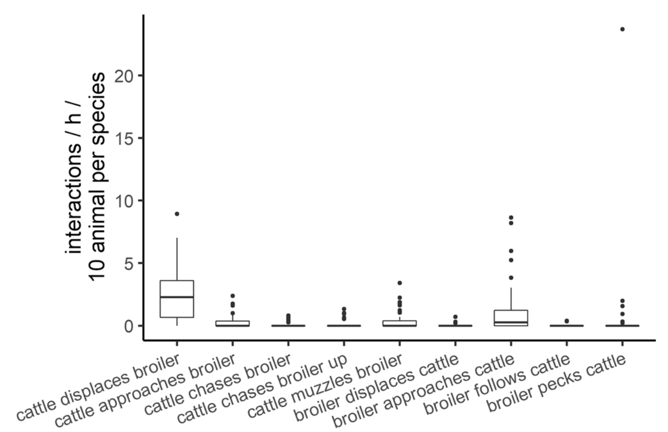

Fig. 5. Number of different inter-species interactions (per hour and ten animals per species) in multi-species groups. Boxplots with median (black line), interquartile range (box), 1.5 x interquartile range (whiskers).

species groups occurred less frequently than intra-species interactions, which could be because of a preference for conspecifics and enough space to allow for a spatial separation of species groups.

Regarding measures of fearfulness, none of the measures of Tonic Immobility that were assessed in four cycles differed between broilers in multi- and single-species groups tested after pasture. Therefore, cattle did not affect fearfulness in broilers as measured by the TI test. If, on the one hand, broilers had perceived cattle as additional enrichment, we would have expected broilers in multi-species groups to be less fearful, indicated by a lower duration in TI (Jones, 1986), compared to broilers in single-species groups, since increased environmental complexity is associated with decreased fearfulness (Jones, 2002; Jones and Waddington, 1992). If, on the other hand, broilers had perceived cattle as something frightening, we would have expected a higher duration in TI for broilers in multi-species groups compared to broilers in single-species groups.

The other two fearfulness tests, Inversion and Novel Object tests, were only performed in one cycle and we are therefore cautious in the interpretation of results. The intensity of the reaction to inversion (wingbeats/s) increased more in broilers in the single-species group than broilers in the multi-species group after pasture, which according to House et al. (2020) could be interpreted as broilers co-grazing with cattle becoming more fearful over the pasture period than those ranging with only conspecifics. However, the response to a novel object did not differ between multi- and single-species broiler groups. In both groups, after six weeks on pasture, broilers approached the novel object less compared to tests before pasture, indicating an age effect which is in line with a study showing that older broilers are less willing to approach a novel object than younger ones (Baxter et al., 2021), but contradicting two other studies (Bailie and O'Connell, 2015; Giersberg et al., 2020). Apart from an age effect, possibly broilers ranging on pasture with or without cattle experienced the pasture as so enriching that an additional novel object was not perceived as worth investigating.

Overall, we tentatively conclude that neither young cattle nor broiler chickens were adversely affected by co-grazing, as the behaviour, including measures of fearfulness, did not differ compared to single-species grazed conspecifics. Whether one or both species experience co-grazing positively or enriching should be investigated in future studies, possibly by including a choice experiment, in which the animals can choose between a pasture with only conspecifics and a pasture with conspecifics and another species.

This first descriptive study yields valuable insights into the behaviour of young cattle and broiler chickens on pasture, based on many hours of observation of both multi- and single-species groups on the same farm simultaneously and under the same management conditions. However, there are methodological aspects worth considering for future studies: First, results should be verified with a larger number of animals per group, preferably similar to the number of animals used on farms to increase the applicability of the results to a commercial context, and a

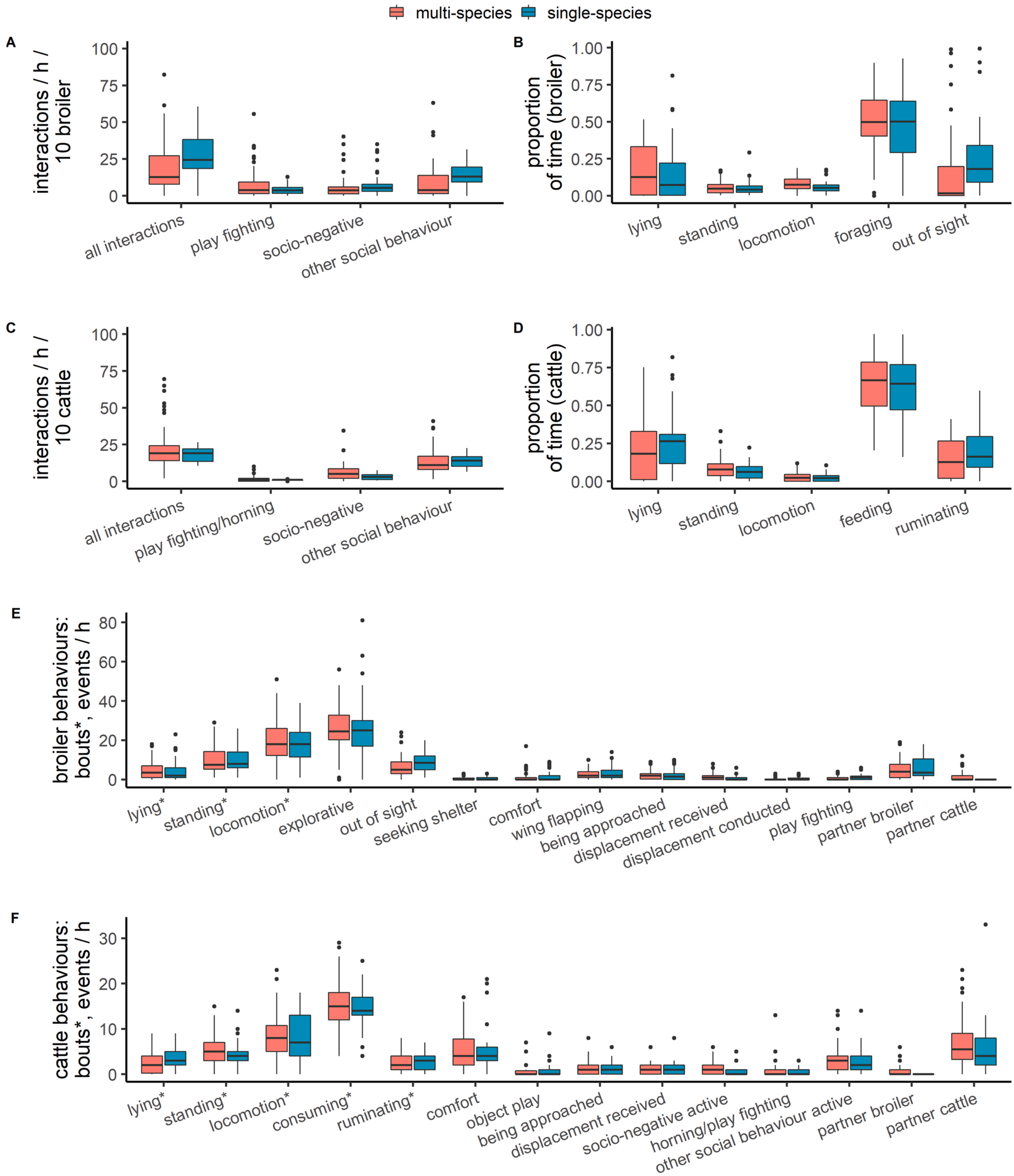

Fig. 6. Results for occurrences of inter-and intra-species interactions (A, C) in single- (turquoise) and multi-species groups (red), as well as proportion of time (recorded as durations) spent performing maintenance behaviours (B, D) and frequency of bouts (marked with *) and events for maintenance, comfort and social behaviour (E, F) (research question 2). Boxplots with median (black line), interquartile range (box), 1.5 x interquartile range (whiskers).

greater number of replicates to allow further statistical analysis. Second, for inter-species interactions to be possible, individuals of the different species have to be close to each other. To measure proximity, we divided the plot into 16 sectors and recorded the number of animals per species per sector. However, since one sector had the size of slightly less than 200 m^2, animals in one sector were not necessarily close to each other. Similarly, animals at the borders of two sectors could be closer to each other than those within a sector, but would be recorded in two different sectors. Future studies could counteract this by using smaller sectors or GPS loggers. Third, the number of observations for single-species cattle

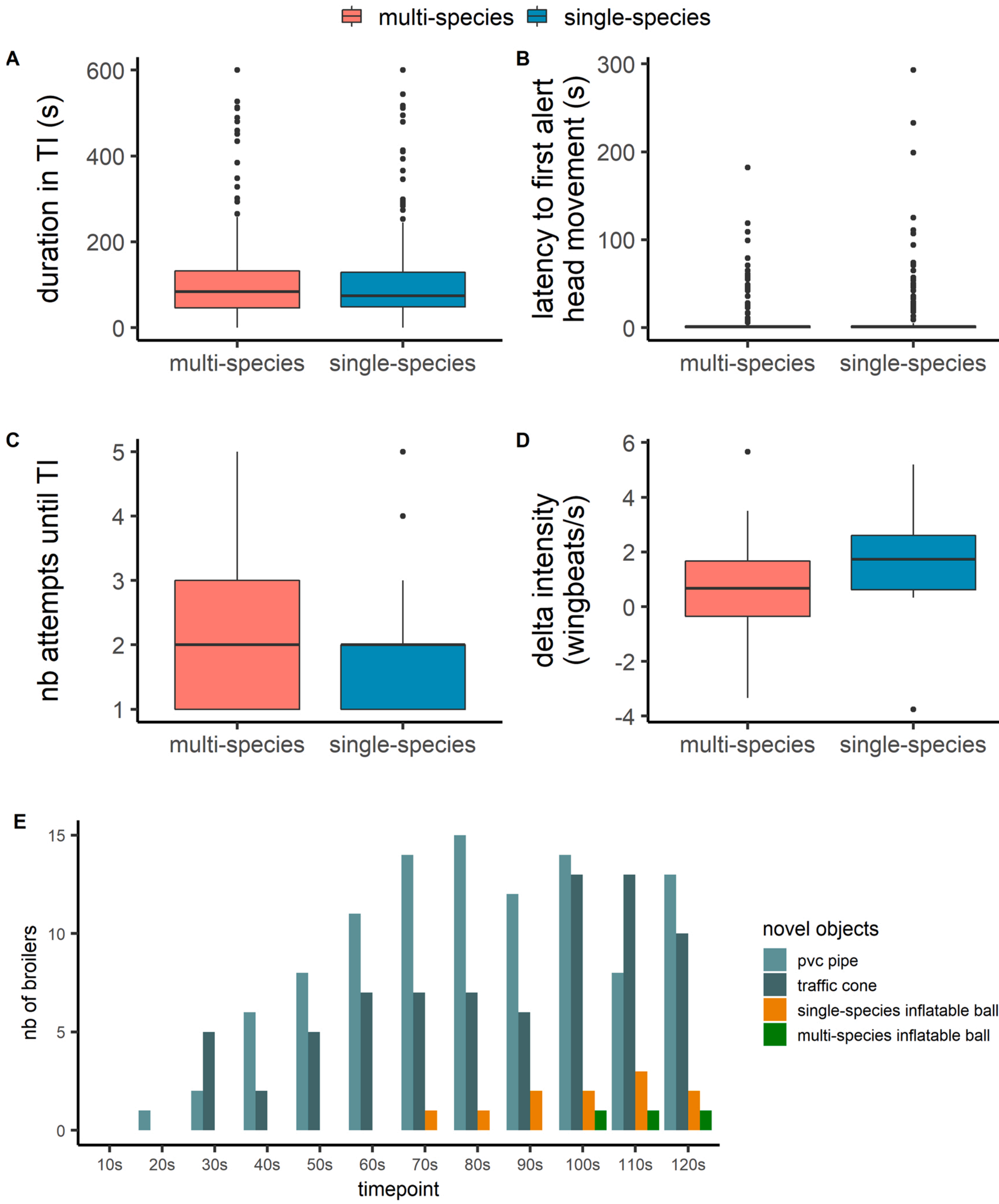

Fig. 7. Results for fearfulness measures in broilers (research question 3), Tonic Immobility (A-C), Inversion test (D) and Novel Object test (E). The duration (s) spent in Tonic Immobility (A), the latency (s) to first alert head movement (B) and the number of attempts necessary to induce Tonic Immobility (C) after the pasture period for single-(turquoise) and multi-species groups (red), the difference (delta: before pasture – after pasture) of the intensity (wingbeats/s) measured for the Inversion test (D) in single- (green) and multi-species groups (red) and the Novel Object test (E) with the number of broilers in a 25 cm radius around the three different novel objects (orange: PVC pipe, green: traffic cone, light blue: inflatable ball in the multi-species group and dark blue: inflatable ball in the single-species group) in 10 s intervals up to 120 s after the object was placed in the pen. (A-D) boxplots with median (black line), interquartile range (box), 1.5 x interquartile range (whiskers). (E) Side-by-side barplot.

groups was low(er) compared to the multi-species groups or the single-species broiler groups. This was due to the rotational grazing system for single-species groups, with the single-species cattle groups starting and finishing the pasture period two weeks ahead of the single-species broiler and multi-species groups, which should be addressed in future experiments. Overall, our experimental design was suitable to generate first insights into the behaviour of co-grazed broilers and cattle.

5. Conclusion

This study on co-grazing young cattle and broiler chickens builds a foundation for future studies investigating behaviour in different multi-species livestock groups. We conclude that neither cattle nor broiler behaviour was affected negatively by co-grazing, thereby indicating no negative welfare consequences. Inter-species interactions such as broiler approaching cattle could tentatively be interpreted as possibly enriching, but further research is necessary to investigate possible positive effects on welfare.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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This manuscript has been released as a Pre-Print at agriRxiv (Schanz et al., 2022).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2022.105779.

References

Anderson, D.M., 1998. Pro-active livestock management capitalizing on animal behavior. J. Arid L. Stud. 7S, 113–116.

Anderson, P.L., Glidden, D.V., Liu, A., Buchbinder, S., Lama, J.R., Guanira, J.V., McMahan, V., Bushman, L.R., Casapía, M., Montoya-Herrera, O., Veloso, V.G., Mayer, K.H., Chariyalertsak, S., Schechter, M., Bekker, L.G., Kallás, E.G., Grant, R. M., 2012. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. Sci. Transl. Med. 4 https://doi.org/10.1126/scitranslmed.3004006.

Archer, G.S., Mench, J.A., 2014. Natural incubation patterns and the effects of exposing eggs to light at various times during incubation on post-hatch fear and stress responses in broiler (meat) chickens. Appl. Anim. Behav. Sci. 152, 44–51. https://doi.org/10.1016/j.applanim.2013.12.010.

Bailie, C.L., O'Connell, N.E., 2015. The influence of providing perches and string on activity levels, fearfulness and leg health in commercial broiler chickens. Animal 9, 660–668. https://doi.org/10.1017/S1751731114002821.

Baxter, M., Richmond, A., Lavery, U., O'Connell, N.E., 2021. A comparison of fast growing broiler chickens with a slower-growing breed type reared on higher welfare commercial farms. PLOS One 16, 1–22. https://doi.org/10.1371/journal.pone.0259333.

Benoff, F.H., Siegel, P.B., 1976. Genetic analysis of Tonic Immobility in young Japanese quail (Coturnix cotunix japonica). Anim. Learn. Behav. 4, 160–162. https://doi.org/10.3758/BF03214027.

R. Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Cuchillo-Hilario, M., Wrage-Mönnig, N., Isselstein, J., 2017. Behavioral patterns of (co-) grazing cattle and sheep on swards differing in plant diversity. Appl. Anim. Behav. Sci. 191, 17–23. https://doi.org/10.1016/j.applanim.2017.02.009.

Daoudi, S., Badihi, G., Buchanan-Smith, H., 2017. Is mixed-species living cognitively enriching? Enclosure use and welfare in two captive groups of tufted capuchins (Sapajus apella) and squirrel monkeys (Saimiri sciureus). Anim. Behav. Cogn. 4, 72–90. https://doi.org/10.12966/abc.06.02.2017.

Dawkins, M.S., Cook, P.A., Whittingham, M.J., Mansell, K.A., Harper, A.E., 2003. What makes free-range broiler chickens range? In situ measurement of habitat preference. Anim. Behav. 66, 151–160. https://doi.org/10.1006/anbe.2003.2172.

De Haas, E.N., Bolhuis, J.E., Kemp, B., Groothuis, T.G.G., Rodenburg, T.B., 2014. Parents and early life environment affect behavioral development of laying hen chickens. PLOS One 9, 34–38. https://doi.org/10.1371/journal.pone.0090577.

Duve, L.R., Weary, D.M., Halekoh, U., Jensen, M.B., 2012. The effects of social contact and milk allowance on responses to handling, play, and social behavior in young dairy calves. Journal of Dairy Science 95 (11), 6571–6581. https://doi.org/10.3168/jds.2011-5170.

Fanatico, A.C., Mench, J.A., Archer, G.S., Liang, Y., Brewer Gunsaulis, V.B., Owens, C.M., Donoghue, A.M., 2016. Effect of outdoor structural enrichments on the performance, use of range area, and behavior of organic meat chickens. Poult. Sci. 95, 1980–1988. https://doi.org/10.3382/ps/pew196.

Forkman, B., Boissy, A., Meunier-salaün, M., Canali, E., Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses 92, 340–374. https://doi.org/10.1016/j.physbeh.2007.03.016.

Gamer, M., Lemon, J., Fellows, I., Singh, P., 2012. irr: Various coefficients of interrater reliability and agreement. R package version 0.84. https://doi.org/https://CRAN.R-project.org/package=irr.

Giersberg, M.F., Poolen, I., de Baere, K., Gunnink, H., van Hattum, T., van Riel, J.W., de Jong, I.C., 2020. Comparative assessment of general behaviour and fear-related responses in hatchery-hatched and on-farm hatched broiler chickens. Appl. Anim. Behav. Sci. 232, 105100 https://doi.org/10.1016/j.applanim.2020.105100.

Gonçalves, S.A., Ferreira, R.A., Pereira, I.G., De Oliveira, C.C., Amaral, P.I.S., Garbossa, C.A.P., Da Silva Fonseca, L., 2017. Behavioral and physiological responses of different genetic lines of free-range broiler raised on a semi-intensive system. Journal of Animal Behaviour and Biometeorology 5 (4), 112–117. https://doi.org/10.14269/2318-1265/jabb.v5n4p112-117.

Hardie, S.M., Buchanan-Smith, H.M., 2000. Responses of captive single- and mixed-species groups of Saguinus to novel nonthreatening objects. Int. J. Primatol. 21, 629–648. https://doi.org/10.1023/A:1005513320601.

House, G.M., Sobotik, E.B., Nelson, J.R., Archer, G.S., 2020. Effect of the addition of ultraviolet light on broiler growth, fear, and stress response. J. Appl. Poult. Res. 29, 402–408. https://doi.org/10.1016/j.japr.2020.01.003.

Hulet, C.V., Anderson, D.M., Smith, J.N., Shupe, W.L., Taylor, C.A., Murray, L.W., 1989. Bonding of goats to sheep and cattle for protection from predators. Appl. Anim. Behav. Sci. 22, 261–267. https://doi.org/10.1007/s10040-008-0378-y.

Jensen, M.B., Kyhn, R., 2000. Play behaviour in group-housed dairy calves, the effect of space allowance. Applied Animal Behaviour Science 67, 35–46. https://doi.org/10.1016/S0168-1591(99)00113-6.

Jones, R.B., 1986. The Tonic Immobility reaction of the domestic fowl: a review. Worlds Poult. Sci. J. 42, 82–96. https://doi.org/10.1079/wps19860008.

Jones, R.B., 2002. Role of comparative psychology in the development of effective environmental enrichment strategies to improve poultry welfare. Int. J. Comp. Psychol. 15, 77–106.

Jones, R.B., Faure, J.M., 1981. Sex and strain comparisons of Tonic Immobility ("Righting time") in the domestic fowl and the effects of various methods of induction. Behav. Process. 6, 47–55. https://doi.org/10.1016/0376-6357(81)90015-2.

Jones, R.B., Waddington, D., 1992. Modification of fear in domestic chicks, Gallus gallus domesticus, via regular handling and early environmental enrichment. Anim. Behav. 43, 1021–1033. https://doi.org/10.1016/S0003-3472(06)80015-1.

Koo, T.K., Li, M.Y., 2016. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J. Chiropr. Med. 15, 155–163. https://doi.org/10.1016/j.jcm.2016.02.012.

Martin, G., Barth, K., Benoit, M., Brock, C., Destruel, M., Dumont, B., Grillot, M., Hübner, S., Magne, M.A., Moerman, M., Mosnier, C., Parsons, D., Ronchi, B., Schanz, L., Steinmetz, L., Werne, S., Winckler, C., Primi, R., 2020. Potential of multi-species livestock farming to improve the sustainability of livestock farms: a review. Agric. Syst. 181, 1–12. https://doi.org/10.1016/j.agsy.2020.102821.

Newberry, R.C., Blair, R., 1993. Behavioral responses of broilers to handling: effects of dietary tryptophan and two lighting regimes. Poult. Sci. 72, 1237–1244. https://doi.org/10.3382/ps.0721237.

Rowden, L.J., Rose, P.E., 2016. A global survey of banteng (Bos javanicus) housing and husbandry. Zoo. Biol. 35, 546–555. https://doi.org/10.1002/zoo.21329.

Savory, C.J., 1976. Broiler groth and feeding behaviour in three different lighting regimes. Br. Poult. Sci. 17, 557–560. https://doi.org/10.1080/00071667608416312.

Schanz, L., Hintze, S., Hübner, S., Barth, K., Winckler, C., 2022. Single- and multi-species groups: a descriptive study of cattle and broiler behaviour on pasture. agriRxiv (preprint) 2022. https://doi.org/10.31220/agrirxiv.2022.00150.

Smith, M.E., Linnell, J.D.C., Odden, J., Swenson, J.E., 2000. Review of methods to reduce livestock depradation: I. Guardian animals. Acta Agric. Scand. A Anim. Sci. 50, 279–290. https://doi.org/10.1080/090647000750069476.

R.Studio Team, 2021. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA. http://www.rstudio.com/.

Ulukan, D., Steinmetz, L., Moerman, M., Bernes, G., Blanc, M., Brock, C., Destruel, M., Dumont, B., Lang, E., Meischner, T., Moraine, M., Oehen, B., Parsons, D., Primi, R., Ronchi, B., Schanz, L., Vanwindekens, F., Veysset, P., Winckler, C., Martin, G., Benoit, M., 2021. Survey data on european organic multi-species livestock farms. Front. Sustain. Food Syst. 5, 1–7. https://doi.org/10.3389/fsufs.2021.685778.

Veasey, J., Hammer, G., 2010. Managing captive mammals in mixed-species communities. In: Kleiman, D.G., Thompson, K.V., Kirk Bear, C. (Eds.), Wild Mammals in Captivity: Principles and Techniques for Zoo Management, 2nd. The University of Chicago Press, Chicago, pp. 151–161.

Valníčková, B., Stěhulová, I., Šárová, R., Špinka, M., 2015. The effect of age at separation from the dam and presence of social companions on play behavior and weight gain in dairy calves. Journal of Dairy Science 98 (8), 5545–5556. https://doi.org/10.3168/jds.2014-9109.

Wallenbeck, A., Wilhelmsson, S., Jönsson, L., Gunnarsson, S., Yngvesson, J., 2016. Behaviour in one fast-growing and one slower-growing broiler (Gallus gallus domesticus) hybrid fed a high- or low-protein diet during a 10-week rearing period.

Acta Agriculturae Scandinavica A: Animal Sciences 66 (3), 168–176. https://doi.org/10.1080/09064702.2017.1303081.

Wang, S., Ni, Y., Guo, F., Fu, W., Grossmann, R., Zhao, R., 2013. Effect of corticosterone on growth and welfare of broiler chickens showing long or short Tonic Immobility. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 164, 537–543. https://doi.org/10.1016/j.cbpa.2012.12.014.

Welfare Quality®, 2009. Welfare Quality® assessment protocol for poultry (broilers, laying hens), Welfare Quality® Consortium, Lelystad, Netherlands.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Winckler, C., Tucker, C.B., Weary, D.M., 2015. Effects of under- and overstocking freestalls on dairy cattle behaviour. Applied Animal Behaviour Science 170, 14–19. https://doi.org/10.1016/j.applanim.2015.06.003.

Yeates, N.T.M., 1963. The activity pattern in poultry in relation to photoperiod. Anim. Behav. 11, 287–289. https://doi.org/10.1016/S0003-3472(63)80112-8.

Zulkifli, I., Che Norma, M.T., Chong, C.H., Loh, T.C., 2000. Heterophil to lymphocyte ratio and Tonic Immobility reactions to preslaughter handling in broiler chickens treated with ascorbic acid. Poult. Sci. 79, 402–406. https://doi.org/10.1093/ps/79.3.402.